RESEARCH NOTE

Evaluation of the BDProbeTec strand displacement amplification assay in comparison with the AMTD II direct test for rapid diagnosis of tuberculosis

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ABSTRACT

The BDProbeTec MTB assay for direct detection of *Mycobacterium tuberculosis* was evaluated in comparison with the AMTD-II assay on 94 samples from different patients with clinical suspicion of tuberculosis. Using a combination of culture on Lowenstein–Jensen medium (with or without preculture in BACTEC 9000) and clinical diagnosis as the standard, BDProbeTec MTB showed high sensitivity and specificity (96.1% and 100%, respectively), similar to AMTD-II (96.1% and 97.1%, respectively), with significantly higher sensitivity than the Ziehl–Neelsen stain for acid-fast bacilli (73%, $p < 0.05$).

Keywords *Mycobacterium*, tuberculosis, diagnosis, IS*6110*, 16S rDNA, strand displacement amplification

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Tuberculosis (TB) remains a global health problem, with one-third of the world's population infected with *Mycobacterium tuberculosis* [1]. Identification of infected patients is still based on staining smears for acid-fast bacilli (AFB) and culturing for mycobacteria, but staining is neither sensitive (40–70% sensitivity in patients with pulmonary TB) nor specific for *M. tuberculosis* [2,3]. Mycobacterial culture combined with biochemical identification provides a specific diagnosis, but requires ≥2–3 weeks [2,3].

In response to the need for a more rapid diagnostic test for TB, various nucleic acid amplification tests have been developed for direct detection of the *M. tuberculosis* complex in respiratory specimens [4–11]. Among these, the Amplified Mycobacterium Tuberculosis Direct test (AMTD; Gen-Probe, San Diego, CA, USA), based on transcription-mediated amplification of *M. tuberculosis*-specific 16S rRNA sequences, was the first molecular assay to be approved by the USA Food and Drug Administration for direct detection of *M. tuberculosis* in respiratory specimens [6,8–10]. More recently, the BDProbeTec MTB assay (Becton Dickinson, Sparks, MD, USA), a semi-automated system based on thermophilic strand displacement amplification [12,13], has been developed. The amplification target is the internal region of IS*6110*, an insertion sequence that exists in multiple copies in the genome of the *M. tuberculosis* complex [14,15]. Both assays have elevated sensitivity (> 90%) and specificity (> 99%) [6,8–10,16–18].

This study evaluated the performance of BDProbeTec MTB for the molecular diagnosis of TB in comparison with AMTD-II, the standard AFB-smear, and bacterial culture on Lowenstein-Jensen solid medium and in BACTEC MYCO/F-Sputa liquid medium (Becton Dickinson). In total, 94 consecutive clinical specimens (69 sputa, 13 urines, five cerebrospinal fluids and seven other extrapulmonary specimens), all from different patients with a high TB suspicion, were collected from January 2000 through September 2001 and submitted to the Clinical Pathology Laboratory, National Institute for Infectious Diseases IRCCS 'L. Spallanzani', Rome, Italy, for detection of mycobacteria. Samples from patients with a previous diagnosis of TB or who were undergoing anti-TB chemotherapy were excluded from the study.

Specimens were liquefied with Sputasol (Oxoid, Basingstoke, UK), concentrated by centrifugation (3000 *g* for 20 min), and decontaminated with Mycoprep (Becton Dickinson). The sediment (*c.* 50 μL) was used to prepare a smear for Ziehl–Neelsen staining [2]. Phosphate-buffered saline was added to decontaminated sediment to give a volume of 2 mL. For culture, 0.5 mL of the suspension was inoculated into a

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40-mL bottle of BACTEC MYCO/F-Sputa liquid medium containing the PANTA/F antimicrobial mixture and SUPPLEMENT/F (Becton Dickinson). Also, two 0.1-mL aliquots were inoculated into duplicate Lowenstein–Jensen tubes. The remainder was stored at −20 °C for batch testing by BDProbeTec MTB and AMTD-II. BACTEC bottles were incubated at 37 °C and monitored for 40 days in a BACTEC 9000 MB fluorometric instrument. BACTEC-positive cultures were cultured on to Lowenstein–Jensen medium before bacterial identification. Lowenstein–Jensen tubes were incubated at 37 °C and examined weekly for 8 weeks. Specimens that yielded *M. tuberculosis* growth on Lowenstein–Jensen medium, either directly or after growth in BACTEC 9000 MB, were considered as culture-positive. Mycobacterial isolates were identified by conventional biochemical tests [2] and the Accuprobe system (Gen-Probe). The BDProbeTec MTB and AMTD-II tests were performed as recommended by the manufacturers. Positive and negative amplification controls were included in each AMTD-II run [6,8,9,12,13,16–18]. Preliminary assessment of both nucleic acid amplification-based assays indicated a lower detection limit of ≥20 *M. tuberculosis* ATCC 25177 cells/mL.

Of the 94 clinical samples from suspected TB patients, 25 yielded *M. tuberculosis* cultures (Table 1); these comprised 21 sputa, one urine, one cerebrospinal fluid and two extrapulmonary specimens. No mycobacteria other than *M. tuberculosis* were found. Of the 25 *M. tuberculosis* culture-positive samples, 19 (76%) were also AFB-smear positive, while two (3%) of the 69 culture-negative samples were AFB-smear positive (Table 1). Considering *M. tuberculosis* culture as the standard for laboratory diagnosis of TB, both molecular tests detected 24 of 25 *M. tuberculosis* culture-positive samples (96% sensitivity for both tests; $p < 0.05$ compared with AFB-smear; Table 1). Similarly, the BDProbeTec MTB and AMTD-II assays had high specificity, yielding positive results for only one (1.4%) and three (4.3%) of 69 *M. tuberculosis* culture-negative samples, respectively.

Concordance analysis indicated that the performances of the BDProbeTec MTB and AMTD-II systems were similar to the standard culture assay ($p > 0.10$; McNemar test, all comparisons). Comparatively, the two amplification assays were similar ($p > 0.10$; McNemar test), and only five of the 94 samples tested gave discordant results between culture and molecular tests. Two of these samples (one sputum and one abscess fluid), for which only the AMTD-II assay yielded positive results, were probably false-positives since other samples from the same patient on different days were negative by all tests, and no clinical evidence of TB was observed during a 1-year follow-up. One culture-negative urine sample was positive by both BDProbeTec MTB and AMTD-II tests, but additional urine samples collected subsequently from the same patient were also culture-positive for *M. tuberculosis*. A low *M. tuberculosis* concentration in this sample might have hampered culture initially, and this result was redesignated as a culture false-negative. Two false-negative results were also obtained in the molecular tests, of which the first was a *M. tuberculosis* culture-positive sputum sample that was also positive only by AMTD-II. The second was a cerebrospinal fluid that was also positive only by BDProbeTec MTB test, raising the possibility that the presence of RNase in this sample may have prevented template amplification by AMTD-II [19]. Notably, the AFB-smear was positive for this last sample.

Overall, the clinical performances of the BDProbeTec MTB and AMTD-II assays appeared to be similar in terms of both sensitivity (96.1% for both tests) and specificity (100% for the former and 97.1% for the latter). Positive and negative predictive values for AMTD-II were 92.6% and 98.5%, with corresponding values for BDProbe-

Table 1. Results of the AFB-smear, AMTD-II and BDProbeTEC MTB in *Mycobacterium tuberculosis* culture-positive and -negative samples[a]

	Number of samples	Number of positive samples[a]		
		AFB-stain	AMTD-II	BDProbetec MTB
M. tuberculosis culture-positive	25	19 (76%)	24 (96%)[b]	24 (96%)[b]
M. tuberculosis culture-negative	69	2 (3%)	3 (4%)	1 (1%)

[a]Results are presented as frequencies and percentages. Frequencies were compared by χ^2 test; concordance between tests was analysed by McNemar's test.
[b]$p < 0.05$ in comparison with AFB-stain.

Tec MTB of 100% and 98.6%, respectively. In the context of this comparative evaluation, it should be highlighted that BDProbeTec MTB offers some technical advantages at the level of automation, internal quality control, sample containment and laboratory safety [6,8,9,12,13,16–18].

In conclusion, the data suggest that the BDProbeTec MTB test is a sensitive and specific tool for direct detection of *M. tuberculosis* in a variety of clinical specimens, including cerebrospinal fluid or urine. However, given the paucity of non-respiratory specimens included in this study, additional testing of such specimens is indicated.

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